



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Hideo Nakao et al  
Serial Number : 304,988  
Filed : September 23, 1981  
For : Cepharosporin derivatives, their preparation  
and compositions containing them  
Group : 122  
Examiner : Paul M. Coughlan, Jr.

*Handwritten signature/initials*  
PB/p

DECLARATION PURSUANT TO 37 CFR 1.132

I, hideo Nakao, a Japanese citizen, residing at 1158, Tomioka-cho, Kanazawa-ku, Yokohama-shi, Kanagawa-ken, Japan, sincerely and solemnly declare and say as follows:

I graduated in March 1955 from the Department of Pharmacy, the Faculty of Medicine, the University of Kyoto. In 1965 I received the Doctor's degree from the said University upon "Synthetic studies on nitrogen-containing azulene series compounds having the analgesic activity". In April 1955 I joined Sankyo Company, Limited, Tokyo, Japan. Since then, I have been engaged mainly in synthesis of organic compounds. I am now the Director of the Chemical Research Laboratories of the said company.

I am a member of Pharmaceutical Society of Japan. I have published a number of scientific papers, including:

"A Novel General method for Synthesizing 7 $\alpha$ - Methoxy-  
Cepharosporins", Tetrahedron Letters, 2705 (1975), and  
"A New Cephamycin Derivative I - Part V", J. Antibiotics,  
29, 969 (1976).

I am one of the applicants of the present application  
serial number 304,988 and conversant with its content, as well  
as content of the reference cited by the Examiner.

The following statements are presented by myself, whereas  
comparative tests regarding biological properties of cephem  
compounds have been performed by biological researchers of the  
said Sankyo Company, Limited.

First of all, I would state that the principal object of  
the invention is to explore novel cephalosporin compounds  
which satisfy triple requirements, namely, (1) strong anti-  
bacterial activity, (2) broad antibacterial spectrum, particularly  
against Gram-positive bacteria including Staphylococcus aureus,  
and (3) capability of being orally administered, which  
requirements having never been met all at once by any of  
prior art cephalosporin compounds.

As shown in the table on page 48 of the specification,  
presently claimed compounds show a superior antibacterial  
activity, in terms of minimal inhibitory concentration,  
against varieties of pathogenic microorganisms, including  
both Gram-positive and Gram-negative bacteria. The MIC values  
of the compounds described therein are indicative of their  
sufficient capability of being used for the treatment of

diseases caused by the pathogenic microorganisms tested.

The strong antibacterial activity of the compounds according to the present invention is, however, only one aspect of the invention because the strong antibacterial activity itself has already been attained by prior art cephalosporins, including Cephalothin, Cephaloridine, Cefazolin and Cefotaxime.

Likewise, the broad antibacterial spectrum that the presently claimed compounds possess has already been attained to a certain extent by prior art cephalosporin compounds.

One of the characteristics of the present compounds is that they show a strong antibacterial activity against Staphylococcus aureus. It is undoubtedly the microorganism , among Gram-positive bacteria, that is representative and most important from the practical aspect. Infectious diseases caused by pathogenic bacteria may be classified into two groups, those caused by Gram-positive bacteria and others caused by Gram-negative bacteria. The proportion of patients infected by the former bacteria is roughly speaking 20 percent for inpatients and 50 percent for outpatients (in Japan), hence chemotherapy with antibiotics of bacterial diseases caused by Gram-positive bacteria is clinically very important. Since the proportion of patients infected by Gram-positive bacteria is significant for outpatients, i.e. 50 percent of those infected and since outpatients are suitably treated only with antibiotics which are administered orally, it is critically important that antibiotics effective against Gram-positive

bacteria, i.e. Staphylococcus aureus, should be effective when administered orally.

Of the triple requirements as noted above, the ability of being administered orally, otherwise stated good absorption from the digestive tract is very important as mentioned above.

When a cephalosporin compound suitable for oral administration by esterifying its carboxyl group at 4-position is administered orally, they are well absorbed by a living body through the digestive tract, then the ester moiety is rapidly hydrolyzed enzymatically, liberating the corresponding carboxylic acid, which is the entity to give the antibacterial effect, in blood at a high concentration. The thus liberated carboxylic acid should be fairly stable so as to ensure the antibacterial effect over a practically long period of time and finally excreted in urine at a high rate. Thus, the recovery rate in urine of an antibiotic when administered orally should be higher than 30 percent, more preferably higher than 40 percent.

Heretofore, a limited number of cephem compounds have been actually employed for oral administration. The representatives are the compounds known for "Cephalexin" and "Cephadrine", each having a phenylglycyl or a dihydrophenylglycyl group at 7-position, a methyl group at 3-position and a carboxyl group at 4-position of the cephem nucleus. Although they are commercially sold and used, they still have some difficulties in respect of a narrow antibacterial spectrum and/or an insufficient antibacterial activity, as will be substantiated by comparative tests given below.

Other cephem compounds such as cephalotin, cephalazolin and cefmetazole, which show an excellent activity against Staphylococcus aureus, for instance, when used by injection, may not be practically used for oral administration. As the present specification teaches on pages 1 and 2, if they are administered orally, only about 5 percent of the dose administered are recovered in urine, thus showing their poor absorption through the digestive tract and their unsuitability for oral administration.

As the second paragraph at page 2 of the specification describes, efforts have been made to improve the absorption of cephalosporin derivatives through the digestive tract by esterifying the 4-carboxyl group. This was motivated by the successful results in the case of penicillins such as "Pivampicillin" and "Bacampicillin".

However, such an attempt turned out to be unsuccessful as shown in the description bridging pages 5 and 6 of the specification in conjunction with the description on page 4 of the specification. Namely, the percentage recovery in urine of compounds (b) and (c) (free acid form), i.e. 1.5 and 2 have been improved only up to 8 and 14 by esterifying the respective compounds with a pivaloyloxymethyl group; the figures revealing their incapability of being administered orally.

Comparative data will be given hereunder in order to demonstrate unobviously advantageous properties of presently claimed compounds.

Test 1.

Antibacterial tests were performed with (a) 1-isopropoxycarbonyloxyethyl 7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate\*; (b) 7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylic acid\*, or Cephalexin. In the case of compound (a), minimal inhibitory concentration (MIC, microgram/ml) was measured 1 hour after treating it with a mouse serum in order to liberate the corresponding free carboxylic acid, i.e. compound (b). This was so done because the tests were performed in vitro, and so, compound (a) had to be converted to the active, free acid form before testing. The results are given in the following table.

\*syn- form

Compound	Microorganisms													
	AA	AA'	BB	BB'	C	D	EE	EE'	FF	FF'	G	H	I	
a	3.1	1.5	0.8	0.8	0.8	>200	0.2	3.1	0.05	50	0.4	3.1	0.4	
b	0.8	0.8	0.4	0.4	0.1	>200	0.1	0.8	≤0.01	100	0.1	1.5	0.4	
c	0.4	1.5	6.2	6.2	3.1	>200	6.2	100	12.5	>200	6.2	>200	200	

In the above table, AA through I denote the following microorganisms:

AA: Staphylococcus aureus 209P

AA': Staphylococcus aureus 56

BB: Escherichia coli NIHJ

BB': Escherichia coli 609

C: Shigella flexneri

D: Pseudomonas aeruginosa

EE: Klebsiella pneumoniae 806  
EE': Klebsiella pneumoniae sp846  
FF: Proteus vulgaris  
FF': Proteus morganii  
G: Salmonella enteritidis G.  
H: Enterobacter sp.  
I: Serratia sp.

It will be evident from the above results that compound (b) exhibits far superior activity against the microorganisms of the genus Klebsiella, Enterobacter and Serratia and some species of the genus Proteus, and a superior activity against some other microorganisms over Cephalexin.

Also it will be evident that compound (a) exhibits an activity almost comparable to compound (b) when treated under the given converting conditions.

#### Test 2.

Antibacterial tests in vivo were performed using mice infected with various pathogenic bacteria and administering a test compound orally to determine ED<sub>50</sub> value. The results are shown in the following table.

Compound	Microorganisms				
	AA'	BB'	EE"	FF"	I'
a	20.00	9.81	1.03	5.26	1.97
c *	50	19.6	19.6	100	100

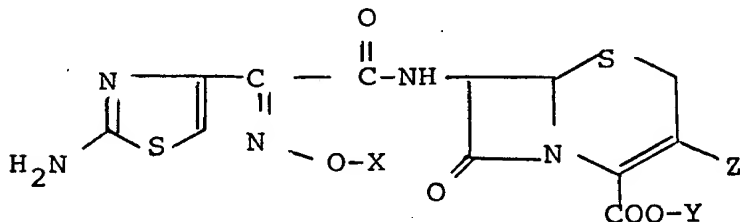
\*Cephalexin

In the above table, compounds (a) and (c) are identical with those in Test 1, microorganisms AA' and BB' are identical with those in test 1, and EE", FF" and I' denote Klebsiella pneumoniae 866, Proteus vulgaris 1413 and Serratia sp.1850, respectively.

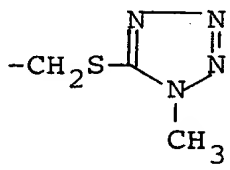
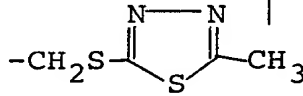
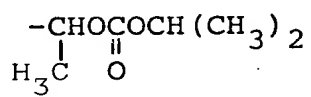
From the above results, it will be evident that compound (a) exhibits far more superior antibacterial activity than Cephalexin in a living body, well suggesting a high conversion rate of compound (a) to compound (b) in vivo.

### Test 3.

In order to assess the biological absorption, each test compound was administered orally to a group of mice, then percentage recovery in urine of the compound until 24 hours after administration was determined. The chemical structure of the compounds and the test results are summarized below.





Compound	X	Y	Z	Recovery rate in urine (%)
d	CH <sub>3</sub>	H		2.0
e	CH <sub>3</sub>	-CH <sub>2</sub> OCOC(CH <sub>3</sub> ) <sub>3</sub>	do	14.0
f	CH <sub>3</sub>	H	-CH <sub>2</sub> OCOCH <sub>3</sub>	1.5
g	CH <sub>3</sub>	-CH <sub>2</sub> OCOC(CH <sub>3</sub> ) <sub>3</sub>	do	8.3
h	H	-CH <sub>2</sub> OCOC(CH <sub>3</sub> ) <sub>3</sub>		14.9
b	CH <sub>3</sub>	H	-CH <sub>2</sub> OCH <sub>3</sub>	4.5
a	CH <sub>3</sub>	-CH <sub>2</sub> OCOC(CH <sub>3</sub> ) <sub>3</sub>	do	75.9
i	CH <sub>3</sub>		do	67.0

It will be evident from the above results that the recovery rate in urine of the compounds d and f may not be improved to such an extent that the corresponding esterified compounds can be used for oral administration. Contrary to this, the compound b may be improved, having regard to the recovery rate in urine, so that the esterified compounds may well be used for oral administration.

It will also be evident that percentage recovery in urine of compounds tested largely varies as the combination of the substituents at 3-, 4- and 7-positions of cephem nucleus changes, that the change in percentage recovery in urine with the change in the chemical structure is quite unpredictable.

Thus, it will fairly be concluded that, even where a particular chemical modification is known to improve the properties of one particular compound, this is not any indication that a similar modification will similarly improve the properties of any other compounds.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information are believed to be true; and further that these statements were made with the knowledge that willful false statements so made are punishable by fine or imprisonment, or both, under 18 USC 1001 and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Declared and signed this 20th day of December 1982  
in Tokyo.



Hideo NAKAO